In re Application of:	Gerald BATIST, et al
Filed:	December 18, 2000
Serial No.:	09/779,223
Title:	HEX II TUMOR-SPECIFIC PROMOTER AND USE THEREOF
Examiner:	Shin Lin CHEN
Group Art Unit:	3120

REMARKS

Rejection of Claims 6 Under 35 USC §101

Claim 6 has been rejected on the basis that the specification fails to assert a substantial and specific utility for the claimed method of tumor-selective expression of a gene in a cell ***in vitro***. Applicant respectfully traverses the Examiner's rejection in this regard, as it is explicitly stated on page 12 lines 12-15 of the present application that the tumor-selective expression of the Hex II promoter has utility in both ***in vivo*** cancer therapy as well as ***in vitro*** laboratory research. An ***in vitro*** use of this aspect of the present invention is specificied at page 12, lines 20-24 wherein it is stated that the Hex II promoter may be employed with a suitable gene operatively linked thereto for screening of tumor cells ***in vitro***. In this manner, the tumor-selective expression of a gene ***in vitro***, according to present invention, allows for easy distinction of tumor cells from normal cells. Applicant believes that this is but one substantial and specific utility for the subject matter of claim 6 on the present application.

Likewise, Applicant points to the teaching of the specification at page 6, lines 4-8 in support of the utility of claim 6. Specifically, the subject matter of claim 6 has utility in screening tumor-specific gene expression ***in vitro***. That is to say, by employing the method of claim 6 it would be readily understood by a person of skill in the art, having read the specification of the present application that this embodiment of the present invention could be readily employed to determine the effects of a particular gene by studying the selective expression of that gene in a tumor cell. In this case, a gene of interest may be a suicide gene for selectively targeting cancer cells with a toxic protein, for example. A further substantial and specific utility of the subject matter of claim 6 is described at page 9, lines 1-14. That is, the use of the claim method for a tumor-selective expression of a gene in a cell ***in vitro*** for testing the tumorigenicity of a cell line.

Accordingly, Applicant submits that the present application teaches at least three substantial and specific utilities for the subject matter of claim 6. Reconsideration and withdrawal of the Examiner's rejections in this regard is respectfully requested.

Rejection of Claims 2, 3, 13-20 and 23-25 Under 35 USC §112 (second paragraph)

Claims 2, 3, 13-20 and 23-25 have been rejected as indefinite for failure to particularly point out and distinctly claim the subject matter therein. Claim 2 is herein in amended to specify a LacX or HSV TK gene. This amendment is believed to overcome the Examiner's rejection thereto.

Applicant respectfully traverses the Examiner's rejections to claims 3, 15-20 and 23-25 alleging that the expression "a basic expression vector" is vague and indefinite, and is not specifically defined in the specification. Basic expression vectors of the present invention are exemplified in Figures 1 & 8 of the specification and described at page 17, lines 13 to page 18, line 15 and at page 22 lines 11-21. According to the these specific teachings of the specification, Applicant submits that a person of skill in the art would readily comprehend the metes and bounds of what is considered "a basic expression vector". However, in order to expedite prosecution, the term "basic" has been deleted from the claims. Reconsideration and withdrawal of the Examiner's rejections in this regard is respectfully requested on this basis.

Claims 13, 19 and 20 have also been rejected as indefinite. Claims 13, 19 and 20 are herein amended to remove references to the Figures appearing therein. Accordingly, the Examiner's rejections in this regard are now believed to be obviated.

Claims 4, 17 and 18 have been rejected as indefinite for failing to include positive steps delimiting how the claimed use is actually practiced. Claims 4, 17 and 18 are herein amended to more explicitly claim a product, and more specifically, a vector for providing selective gene expression in a

tumor cell. In view of these amendments, Applicant respectfully submits that the Examiner's rejections in this regard are herein also obviated.

Rejection of Claims 5, 7-12 Under 35 USC §112 (first paragraph)

The Examiner has rejected claims 5 and 7-12 of the present application on the basis of a lack of enablement for the subject matter thereof in the specification. Applicant respectfully traverses the Examiner's rejections in this regard.

Firstly, Applicant draws the Examiner's attention to the fact that the rejected claims pertain to a method for a tumor-selective expression of a gene in a cell, whereby a rat Hex II promoter is selectively activated in tumor cells as compared with normal cells and NOT to a method of "killing" tumor cells. Although, tumor cell death could effectively result from practicing the method as claimed, claims 5 and 7-12 are not directed thereto, and accordingly Applicant respectfully traverses the Examiner's rejections in this regard, as inapplicable.

Applicant submits that the present application clearly enables the scope of these claims, as particularly evidenced in respect of the data disclosed in connection with Example I of the present application. Example I of the present application provides support for the ***in vivo*** gene transfer and tumor-selective expression of the Hex II promoter in a mouse mammary carcinoma model and a human lung carincoma model (Figures 6A-6E and 7). Specifically, Example I illustrates that due to an increase in rat Hex II promoter activity (i.e. selective activiation) in tumor cells as compared to normal cells, a reduction in tumor volume ***in vivo*** was achieved when DA-3 mice when treated with gancyclovir (GCV) intratumoral injection and AdHexTk, AdHexLac and AdHexRSVTK, respectfully. Applicant respectfully submits that this evidence provides adequate guidance to a person of skill in the art that a tumor-selective expression of a gene under the control of a rat Hex II promoter would provide sufficient expression of a gene product to obtain a therapeutic effect ***in vivo*** for a particular cancer cell.

In addition, the evidence presented in connection with Example II of the present application provides additional support for the method of the rejected claims, namely the tumor-selective expression of gene in a cell (Figures 9B and 9C), selective promoter activation (Figures 11A-11I); the use of adenovirus vectors in achieving selective toxicity in tumor cells vs. normal cells (Figure 12B & Table 1). Although the support as provided in Example II pertains largely to ***in vitro*** data, Applicant respectfully submits that this evidence is complimentary to the ***in vivo*** evidence provided in respect of Example I and would be considered by a person of skill in the art to substantiate the ***in vivo*** aspects of the claimed method. In particular, Applicant believes that this evidence, when considered with the teachings of the specification as a whole, provides adequate guidance and support for the combination of a toxic gene, such as HSV Tk gene or Cytochrome P450 gene, and a prodrug under the control of a rat Hex II promoter to provide sufficient expression of the gene product to inhibit tumor cell growth, as claimed, ***in vivo***. Applicant also points out that additional support for the use of a Cytochrome P-450 2B1 gene and corresponding prodrugs cyclophosphamide, penicillin, amidase and ß-lactamase can be found of page 12 of the specification.

As detailed hereinabove, Applicant believes that the subject matter of rejected claims 5 and 7-12 are fully enabled by the teachings of the specification to the extent that a person of skill in the art would be able to make or use the invention commensurate in scope with the present claims. That is, the teachings of the specification provide sufficient enablement to allow a person of skill in the art to practice a method of tumor-selective expression of a gene under the control of a rat Hex II promoter both ***in vivo*** and ***in vitro***, as claimed in the present application.

The Examiner has raised further rejections to claims 5 and 7-12 on the basis that the state of the art for gene therapy was unpredictable at the time of the invention, thus rendering a requirement for undue experimentation by one of skill in the art in order to practice the invention as claimed therein. Again, Applicant respectfully traverses the Examiner's rejections in this regard.

Firstly, the Examiner points to the teachings of Deonarain and Verma which emphasis that the biggest problems hampering successful gene therapy is the the ability to target a gene to a significant population of cells and express it at adequate levels for a long enough period of time. However, Applicant submits that the nature of the present invention overcomes these problems, in that the rat Hex II promoter is tumor selective and a corresponding construct of the present invention will selectively activate a gene contained therein in a targeted fashion, namely only in tumor cells.

Secondly, the use of the present invention in the context of gene therapy would have distinct advantages over conventional gene therapy methods and/or techniques in that treatment can be focused in the immediate region of the cancer, and the tumor-selective promoter would ensure that a high copy number of vectors, for example, could be used in a small treatment area without risk of toxicity to non-cancerous (normal) cells. Furthermore, high-level or long-term expression is unnecessary because tumor cells would be irradicated with the expression of a relatively small amount of toxin in comparision with the level of expression required in traditional gene therapies. Accordingly, Applicant respectfully submits that undue experimentation would NOT be required by a person of skill in the art in order to practice the invention as claimed in claims 5 and 7-12. Favourable reconsideration of the Examiner's rejections in this regard is respectfully requested on the basis of Applicant's arguments set forth hereinabove.

Rejection of Claims 1-4, 15 and 18 Under 35 USC §102

Claims 1-4, 15 and 18 of the present application have been rejected as anticipated in view of the teachings of Mathupala *et al.* Applicant respectfully traverses the Examiner's rejections in this regard.

Claim 15 is herein amended to clarify and consistently claim a rat Hex II promoter that is "selectively activated in tumor cells as compared with normal cells".

Specifically, Mathupala *et al.* ((1995) J. Biol. Chem. 270, 16918-16925) reported the isolation and sequencing of the rat Hex II promoter from rapidly growing, highly glycolytic hepatoma cell line (AS-30D). In addition, Mathupala *et al.* reported enhanced promoter activity in the rat tumor cell line (AS-30D) as compared with transfected rat hepatocytes, in the presence of modulators of interest, i.e. glucose, insulin and glucagon. As discussed at page 16924, col. 2, Mathupala et al. suggest that the failure of the rat Hex II promoter to be activated in hepatocytes by the common modulators suggests that a different set or level of transcription factors may be involved in normal and transformed hepatocytes for controlling the expression of hexokinase isoforms and therefore the rate of glucose catabolism. As concluded on page 16925, the studies of Mathupala et al. suggest that differences in the regulation of hexokinase genes involved in glucose catabolism appear between normal versus tumor cells in rat.

Although increased levels of hexokinase type II isoform were noted in rat tumor cells as compared to rat normal cells, no evidence was presented to indicate that the rat Hex II promoter itself, was in any way tumor specific. Mathupala et al. do not teach of the transformation of cells of other species with a construct including the rat Hex II promoter, and accordingly do not teach or suggest that a rat Hex II promoter is selectively activated in tumor cells in general. In fact, Mathupala et al. do not even suggest that a similar correlation, that is, a difference in the regulation of hexokinase genes between normal and tumor cells, would be expected in any species other than rat.

Accordingly, Applicant believes that the tumor-specific gene construct of claims 1-4, 15 and 18 patentably distinguish from the teachings of Mathupala et al. on the basis that they are infact tumor-specific. Mathupala et al. do not teach of a Hex II promoter that is selectively activated in tumor cells versus normal cells, nor do they teach of a tumor-specific gene construct. Applicant respectfully submits that the mere observation of enhanced promoter activity in rat tumor cells is quite different from

establishing the utility of a true tumor-specific promoter. Favourable reconsideration and withdrawal of the Examiner's rejections on the basis of anticipation by Mathupala et al. is respectfully requested.

Rejection of Claims 1-6, 8-20 and 23-25 Under 35 USC §103

The Examiner has rejected claims 1-6, 8-20 and 23-25 as obvious in view of several prior art references. In particular, the Examiner has suggested that a person of ordinary skill in the art at the time the present invention was made would have been motivated to substitute the rat cells (as taught by Mathupala *et al.*) with human cells (as taught by Adams) to study the activity of the rat Hex II promoter in normal human cells and human tumor cells based on the finding that rat Hex II promoter activity was increased in rat tumor cells versus normal rat cells (Mathupala *et al.*).

Applicant respectfully traverses the Examiner's rejections in this regard.

Applicant once again points out that Mathupala *et al.* merely teach of a difference in the regulation of hexokinase genes involved in glucose catabolism between normal and tumor cells in rat only, and the sequencing of the rat Hex II promoter. This teaching does not in any way teach or suggest that the rat Hex II promoter is tumor-specific, nor does it suggest that similar differences in the regulation of hexokinase II gene expression would be evident in cells other than rat cells.

In comparision, the present invention is directed to a **tumor-selective** gene construct comprising a rat Hex II promoter that is selectively activated in tumor cells as compared with normal cells, in general. As illustrated in the present application, and argued hereinabove, the tumor-specificity of the rat Hex II promoter has been proven in human and non-human tumor cells. Applicant submits that the data provided in conjunction with the present application supports the finding that the rat Hex II promoter is in fact tumor-specific, and its activity has been shown in a variety of both human and murine tumor cells (as exemplified in Example I & II). As such, the selectivity of the rat Hex II promoter has

been proven by the present invention and Applicant contends that this finding was not suggested in the prior art, nor an obvious extension thereof.

Considerable experimentation was required to test the activity of a non-human promoter sequence in human and murine cells. Based on the extensive experimentation employed in arriving at the present invention, as outlined in the detailed description portion of the present application it was determined that the rat Hex II promoter is activated in a variety of transformed tumor cell lines and not in non-transformed normal cells. Applicant believes that the extensiveness of the experimental effort employed in accordance with the present invention is evidenced in the specification of the present application, and supports the non-obviousness of the claims contained therein. For example, Applicant's experimentation included studying the gene construct of the present application *in vitro* in non-small lung carcinoma H661 and H460 cell lines, human mammary carcinoma MCF-7 cell line and *in vivo* in a mouse mammary carcinoma model and human lung carcinoma model where NCI-H661 cells were grown as a subcutaneous tumor in nude mice. As a result, Applicant arrived at the surprising and unexpected conclusion that the activity of rat Hex II promoter is in fact tumor-specific in tumor cells, in general.

Furthermore, presentation of the present invention was received with enthusiasm by the scientific community. Thus providing further evidence of this surprising and unexpected determination. In view of Applicant's determination that the rat Hex II promoter is tumor cell specific, the therapeutic and experimental utility of this promoter in both animal and human cells became evident.

The unexpected success of the present invention also contradicts the the Examiner's contention that the state of the art for gene therapy was generally unpredictable and inefficient at the time of the present invention, and further serves to support the inventiveness or unobviousness of the present invention.

In re Application of:	Gerald BATIST, et al
Filed:	December 18, 2000
Serial No.:	09/779,223
Title:	HEX II TUMOR-SPECIFIC PROMOTER AND USE THEREOF
Examiner:	Shin Lin CHEN
Group Art Unit:	3120

Applicant respectfully submits that the present invention provides a new and unobvious tumor-specific gene construct comprising a rat Hex II promoter wherein the promoter is selectively regulated in tumor cells as compared to normal cells, and a related method for tumor-selective expression of a gene. Based on the inventive feature, that the claimed gene construct is tumor-specific, all subsequently dependent constructs and methods are also believed to have patentable merit over the teachings of the prior art.

Favourable reconsideration of the Examiner's rejections in this regard is respectfully requested.

Accordingly, Applicant respectfully submits that the present invention patentably distinguishes over the teachings of the prior art, is fully enabled by the teachings of the specification and clearly and definitely claims the invention disclosed in a manner that is deserving of patentable merit commensurate with the scope of the claims presently on file.

Pages showing changes to the above-noted claims set forth above are attached hereto.

A Petition for a One-Month Extension of time and corresponding fee are attached hereto. If any fee deficiencies are associated with this filing the Commissioner is authorized to charge the NIXON PEABODY LLP Deposit Account No. 50-0850.

Date: 4/18/03

Respectfully submitted,

Customer No.: 26770

David S. Resnick (Reg. No. 34,235)
NIXON PEABODY LLP
101 Federal Street
Boston, MA 02110
(617) 345-6057

VERSION TO SHOW MARKED CHANGES TO CLAIMS

2. (Amended) The gene construct of claim 1, which further comprises a LacZ or HSV Tk gene.

3. (Amended) The gene construct of claim 1, wherein said vector is selected from one of a [basic] expression vector, a shuttle plasmid, an adenovirus type 5 recombinant vector or a lipid based delivery system.

4. (Amended) A vector for providing [use in] selective gene expression in a tumor cell, said vector comprising a rat Hex II promoter that is selectively activated in tumor cells as compared with normal cells.

13. (Amended) The gene construct of claim 1, wherein said construct is pHexII4557-CAT. [as set forth in Fig. 1.]

15. (Amended) A tumor-specific Hex II gene construct comprising a rat Hex II promoter operatively linked to a gene and a vector selected from one of a [basic] expression vector, a shuttle plasmid, an adenovirus type 5 recombinant vector or a lipid-based delivery system wherein the promoter is selectively activated in tumor cells as compared with normal cells.

17. (Amended) The tumor-specific Hex II gene construct of claim 3, wherein the tumor cell is a human tumor cell. [for use in selective expression of a gene in human tumor cells].

18. (Amended) The tumor-specific Hex II gene construct of claim 15, wherein the tumor cell is a non-human tumor cell [for providing use in selective expression of a gene in non-human tumor cells].

19. (Amended) The gene construct of claim 15, wherein said vector is pΔE1sp1B and said construct is pΔE1sp1BHex-LacZ. [as set forth in Fig. 2.]

20. (Amended) The gene construct of claim 15, wherein said vector is pΔE1sp1B and said construct is pΔE1sp1BHex-TK. [as set forth in Fig. 3.]

21. [Withdrawn from consideration]

22. [Withdrawn from consideration]